

Claims

1. A method of protecting a plant or a part of said plant against insect or nematode infestation by one or more insects or nematodes having digestive cysteine proteases, comprising presenting to a locus wherein said insect(s) or nematode(s) is (are) to be controlled an inhibitory amount of a cysteine protease inhibitor selected from the group of proteins containing at least one type I repeated thyroglobulin domain.

2. The method of claim 1, wherein the insects have cysteine proteases that are insensitive to host plant derived cysteine protease inhibitors.

3. The method of claims 1 or 2 wherein the insects are one or more of Colorado potato beetle, corn rootworm, thrips and leafminer.

4. The method of claim 1, wherein the nematodes are cyst nematodes or root knot nematodes.

5. The method of any claims 1 to 4, wherein the protein containing at least one type I repeated thyroglobulin domain is human p41 invariant chain fragment or a homologue or functional derivative thereof.

6. The method of any of claims 1 to 4, wherein the protein containing at least one type I repeated thyroglobulin domain is isolated from the sea anemone *Actinia equina* and having the amino acid sequence set forth in figure 1 or a functional derivative or a homologue thereof.

7. The method of any of claims 1 to 4, wherein the protein containing at least one type I repeated thyroglobu-

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lin domain is a protein isolated from the eggs of chum salmon or a homologue or functional derivative thereof.

5 *Sub 3*
8. The method of any of claims 1 to 7, comprising inserting into the genome of the plant a sequence coding for a protein containing at least one type I repeated thyroglobulin domain with a promoter sequence active in the plant to cause expression of said protein at levels which provide an insect or nematode controlling amount of said protein.

10 9. A method according to claim 8, further comprising the steps of:

(a) culturing cells or tissues from the plant;

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(b) introducing into the cells or tissue at least one copy of a gene coding for the protein containing at least
15 one type I repeated thyroglobulin domain;

(c) regenerating resistant whole plants from the cell or tissue culture.

20 10. The method according to claim 9, which comprises the further step of sexually or clonally reproducing the whole plant in such a manner that at least one copy of the sequence coding for the protein containing at least one type I repeated thyroglobulin domain with a promoter sequence active in the plant is present in the cells of progeny of the reproduction.

25 11. The method according to claim 10, further comprising the steps of:

Sub 4
(a) selecting a fertile, insect or nematode resistant plant prepared by the method of claim 10;

30 (b) sexually crossing the insect or nematode resistant plant with a plant from the insect or nematode susceptible plants from the susceptible variety;

(c) recovering reproductive material from the progeny of the cross and

(d) growing resistant plants from the reproductive material.

5 12. The method according to claim 11, for imparting insect or nematode resistance to a substantially homozygous population of plants of a susceptible variety, which comprises the further steps of repetitively:

10 (a) backcrossing the insect or nematode resistant progeny with substantially homozygous, insect or nematode susceptible plants from the susceptible variety; and

(b) selecting for expression of both insect or nematode resistance and the other characteristics of the susceptible variety among the progeny of the backcross, until the desired percentage of the characteristics of the susceptible variety are present in the progeny along with the insect or nematode resistance.

13. A transgenic plant and its sexual progeny resistant to attack by one or more insects or nematodes having digestive cysteine proteases, said transgenic plant expressing an insect or nematode controlling amount of a protein containing at least one type I repeated thyroglobulin domain.

14. A biologically functional expression vehicle
25 containing a promoter effective to promote expression of a
downstream coding sequence in plant cells, a DNA coding
region coding for the expression in plant cells of protein
composed of at least one type I repeated thyroglobulin
domain and a termination sequence effective to terminate
30 transcription or translation of the genetic construction
product in plant cells, the genetic construction effective
to express in the cells of the plant insect controlling
amounts of the protein containing at least one type I
repeated thyroglobulin domain.

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15. The biologically functional expression vehicle of claim 14, wherein the DNA isolate encodes an amino acid sequence set forth in Figure 1 or a functional derivative thereof.

16. The biological functional expression vehicle of claim 14, wherein the expression vehicle is pCAB1.

17. A host cell transformed with a biologically functional expression vehicle of any one of claims 14 to 16.

10 18. The transgenic host cell of claim 17, wherein the DNA sequence is controlled by a promoter effective to promote expression of a downstream coding sequence in a plant cell, the DNA sequence coding region for the expression in plant cells of protein composed of at least
15 one type I repeated thyroglobulin domain and a termination sequence effective to terminate transcription or translation of the product in plant cells, the genetic construction product in plant cells, the genetic construction effective to express in the cells of the plant
20 insect controlling amounts of the protein composed of at least one type I repeated thyroglobulin domain to control one or more insects having digestive cysteine proteases.

19. The method of any of claims 1 to 7, wherein an agricultural composition containing a carrier and an insect
25 or nematode controlling or combatting amount of the cysteine protease inhibitor is applied to the locus.

20. An agricultural composition containing a carrier and an insect or nematode controlling or combatting amount of a cysteine protease inhibitor as defined in claims 1, 5,
30 6 or 7 as an active ingredient.

21. A type I repeated thyroglobulin domain inhibitor peptide with activity towards aspartic proteases, said peptide having the amino acid sequence extending from amino acid position 68-199 of equistatin of Fig. 1 or a modified

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type I repeated thyroglobulin aspartic protease inhibitor peptide wherein said modified peptide comprises a peptide having substantial amino acid identity to amino acid position 68-199 of equistatin; truncations of amino acid
5 position 68-199 of equistatin; or truncations of the peptide having substantial amino acid identity to amino acid position 68-199 of equistatin, wherein said modified peptide is functionally equivalent to said amino acid position 68-199 of equistatin with aspartic protease
10 inhibitor activity.

22. A method of protecting a plant or a part of said plant against insect or nematode infestation by one or more insects or nematodes having digestive aspartic proteases, comprising presenting to a locus wherein said insect(s) or
15 nematode(s) is (are) to be controlled an inhibitory amount of an aspartic protease inhibitor as defined in claim 21.

23. A method of protecting a plant or a part of said plant against insect or nematode infestation by one or more insects or nematodes having digestive cysteine and aspartic
20 proteases, comprising presenting to a locus wherein said insect(s) or nematode(s) is (are) to be controlled an inhibitory amount of an aspartic protease inhibitor as defined in claim 21 and a cysteine protease inhibitor selected from the group of proteins containing at least one
25 type I repeated thyroglobulin domain.

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